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BIOLOGICAL BULLETIN

ARTIFICIAL REMOVAL OF THE GREEN BODIES OF HYDRA VIRIDIS.

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Whether the green bodies in hydras are infecting algæ or products of their own metabolism has been long disputed. I have discovered a method by which they may be removed and the hydras continue to live nevertheless and to multiply.

Semper suggested that these green bodies in *Hydra viridis* might be algæ. A little later Brandt made a careful study of them and concluded that they are algæ which probably live parasitically in the endoderm cells of the hydra.

At the same time Lankester also studied these green bodies. He said: "It appears to me that an examination of the green colored corpuscles of *Hydra* demonstrates those corpuscles to be similar in nature to the chlorophyll bodies of green plants, and that there is no more reason to regard them as symbiotic algæ than there is to regard the green corpuscles in the leaf of a buttercup as such."

Sallitt examined the green bodies in several species of Protozoa and found them to be identical with the green bodies of *Hydra* and *Spongilla*. This uniformity of the green bodies in different animals and their similarity to the chloroplasts of plants led to the belief on the part of a few zoölogists that the green bodies in animals and plants are identical and probably have the same function in both kinds of organisms.

Beyerinck in 1890 isolated the green bodies from *Hydra viridis* and succeeded in making pure cultures of them in an artificial medium. This demonstrated that the green bodies were different from the chloroplasts of plants. He, moreover, identified them with the alga, *Chlorella vulgaris*.

Various theories have been advanced to account for the presence and function of this green alga in animal cells. Some workers have maintained that these cells assimilate the CO_2 which is given off by the animal cells, and then in their turn give off oxygen which is used in the life processes of the animal cells. Others have imagined that the algæ might manufacture products in the presence of sunlight which are passed out into the animal cells and used by them as a food.

Instead of a translocation of the algal reserves to the animal tissues Famintzin and Beyerinck have shown that the alga itself is liable to absorption and digestion by the host. Gamble and Keeble found that mature and immature *Convoluta roscoffensis* digest masses of their own green cells and that the animals obtain little if any food by the translocation of the reserves of its green cells.

They also found that the alga is not transmitted through the egg to the following generation as in the case of *Hydra viridis*, but that the young embryos of each generation are infected by the alga as soon as they leave the egg. Furthermore, "the relation between animal and green cells is a complex one, and cannot be described as symbiotic. The green cell once in the body of the animal probably never escapes; either it is digested or it dies when the animal dies."

In the winter of 1905–6 while keeping *Hydra viridis* in various chemical solutions in order to find some means of causing the development of the reproductive organs it was discovered that animals kept in a weak solution of glycerine lose their green color.

A series of experiments was carried on at that time and in the following winter and spring under the direction of Prof. T. H. Morgan and Prof. W. J. Gies. The following data will show the nature of the results obtained:

Experiment I.— February 19, 1906. Temperature 20°C. Several green hydras were put into a 1.25 per cent. solution of glycerine without food.

February 26. Only 3 were alive, and appeared white to the ordinary eye. These were placed in spring water without food.

March 3. The 3 hydras were beginning to become green in color at the oral end.

March 23. Hydras had normal green color.

Experiment II.— March 1. Temperature 20° C. Many green hydras put into a .5 per cent. solution of glycerine.

March 1. One hydra which appeared white to the eye, but which showed a single green patch of algæ in one tentacle under the lens, was isolated in spring water without food.

March 18. Green color had redeveloped gradually and was at this time identical with that of an ordinary hydra.

Experiment IV.— April 16. Temperature 20° C. Many green hydras put into a .5 per cent. solution of glycerine without food.

April 30. Four hydras which showed no green color under the lens were isolated in spring water without food.

May 7. The hydras showed no trace of green color under the lens. Experiment discontinued.

Experiment VIII. — May 5. Temperature 20° C. Many green hydras put into a .5 per cent. solution of glycerine. Fed every 72 hours with rotifers, *Hydatina senta.* Several formed buds in the glycerine solution.

May 24. Nine hydras which showed no trace of green color under the lens were isolated in a .25 per cent. solution of glycerine and the feeding continued. One individual had a bud attached which itself was budding. None of the others had buds.

May 26. One of the other 8 individuals was budding.

May 27. Three of the 8 individuals were budding.

May 29. Buds had become detached from two of the hydras. Experiment discontinued.

Experiment X. — March 12, 1907. Temperature 20° C. Many green hydras were put into a .5 per cent. solution of glycerine without food.

March 27. Began to add food every 24 hours.

April 7. Isolated 30 hydras which showed no trace of green color under the lens in small glasses containing spring water.

April 23. None had budded. Some had died and some were developing green color.

April 25. Fifteen hydras alive. Nine showed no trace of green color under lens and six showed green patches of algæ scattered in various parts of the body.

Lot A. April 25. Put three of the white hydras into a large balanced aquarium. Food added every 24 hours.

April 27. The three hydras were larger and each had one bud attached.

April 28. The buds had become detached from parent hydras.

April 30. The buds were larger than when detached. Each of the 3 parent individuals was budding again.

May 1. One of the parent hydras had two buds attached.

May 2. Nine individuals. Two of the parent hydras were budding. None showed a trace of green color under the lens.

May 5. Eleven individuals.

May 8. Twelve individuals, 5 of which were budding.

May 10. Sixteen individuals, 4 of which were budding, one had 2 buds. None showed a trace of green color under the lens.

May 27. Many white hydras on lighted side of aquarium.

Lot B. April 27. Put 6 white hydras into another large balanced aquarium which contained green hydras. The white hydras were not in very good condition ; they were fed daily with rotifers.

May 1. Only two white hydras alive.

May 13. Several white hydras seen on walls of aquarium in the midst of the green ones. Some individuals were budding.

May 27. Many white hydras on the lighted side of aquarium.

Experiment XII.—April 4. Put one *Hydra fusca* together with many *Hydra viridis* into a .5 per cent. solution of glycerine without food.

April 14. Food added.

May 1. Twelve of the *Hydra viridis* had lost all green color. *Hydra fusca* was reddish orange in color. It had produced several buds.

May 8. Eight *Hydra fusca*, three of which were budded. Much larger and very different in color from the *Hydra viridis* that had lost their green color.

May 13. Thirteen *Hydra fusca*. Two individuals had 1 bud, 3 had 2 buds and 2 had 3 buds attached.

Experiment XIII. — April 14. Put several green hydras into a .5 per cent. glycerine solution. Food added daily.

May 10. Some hydras had no visible green color and were rather small in size.

Lot A. May 10. Put 5 of the white hydras into spring water without food.

May 20. All alive and white. Four were on the lighted side of the jar.

May 27. Five hydras alive. Two showed no green color but 3 had small green patches around the oral end.

Lot B. May 10. Put 6 of the white hydras into spring water without food.

May 20. All alive and white. Three were on lighted side of jar and 3 were on the bottom of the jar.

May 27. Four hydras alive. One showed no green color but 3 had small green patches around the oral end.

The action of the glycerine upon the green hydra is to cause the algal cells to leave the entoderm cells and to pass into the digestive cavity, from which they are expelled to the outside through the mouth when the hydra contracts. In many instances when hydras were examined which had been in the glycerine solution for a few days, masses of the green algæ could be seen in their digestive cavities. In one case the expulsion of the algæ was actually seen. In other cases no masses of the algæ were ever seen. However, the bottom of the glass upon which the animals were usually located always became more or less green within a small radius of each individual, showing that the algæ were expelled and sank to the bottom of the dish. The algæ never increased to any noticeable extent, but apparently died.

It seems evident from these experiments and observations that the alga in *Hydra viridis* does not play a very important rôle in the life processes. The animal is able to live many days in the glycerine solution without food while in the process of losing its algæ. In Experiments IV. and X. the hydras were without food for about 14 days, and in other experiments the animals have appeared to be in a normal condition, except smaller in size, when kept in glycerine solution without food for 21–30 days. Experiments IV. and XIII. also show that hydras that have lost all their green color can live at least for 7–17 days in spring water without food and at the end of this time be in a normal condition. Very likely they can live a much longer time than this without food.

The loss of the algæ does not seem to interfere with the process of bud formation in the slightest degree. Experiments VIII. and X. show that if the white hydras are fed sufficiently they will produce buds at a normal rate and in a normal manner.

It is a well-known fact that green hydras will live several weeks without food but of many hydras that I have kept 2–5 weeks in various experiments without food none ever produced buds, thus showing that the alga does not furnish food enough for bud formation. However, if sufficient food is given to either the white or green hydras buds soon appear.

The hydras from which all algæ have been extracted do not become reinfected with the algæ. In Experiment X. both lots of the white hydras were kept in large balanced aquaria in which there were hundreds of green hydras, but they remained white. Furthermore the white hydras were fed upon rotifers, the digestive tract of which was usually filled with *Euglena viridis*. It will be recalled that Sallitt believed that the green bodies of *Euglena* were identical with those of the green hydra. However, the colorless hydras are not infected by even this contaminated food supply.

The reappearance of the algæ in some of the supposed white hydras can be readily explained by the supposition that all of the algæ were not removed from the endoderm cells of the hydras before they were transferred to spring water. When the animals were placed again in their normal environments the alga began to grow and reproduce itself until the hydras became as green as normal ones.

The white hydras seems to respond to the stimulus of light in the same manner as the green hydras. In Experiment XIII., where the hydras were starved, some of them collected on the lighted side of the dish and the others remained upon the bottom. None were found on the least illuminated side of the dish.

In Experiment X., lot A, the white hydras were suspended in the large aquarium in a small dish. They did not leave the dish, but climbed upon its lighted side. The food, *Hydatina senta*, shows no reaction to light, so that it cannot be supposed that the hydras moved towards a food supply.

Some of the white hydras of lot B in the same experiment left the

small dish, which was suspended in another large aquarium about three inches from the lighted side, made their way to the lighted side and there became attached. Those that remained in the small dish collected upon its lighted side.

Some of the early workers suggested that *Hydra viridis* might be a variety of *Hydra fusca* which had acquired the power of harboring green algæ in its cells. Greenwood and others found that the endoderm cells of green hydras contain brownish colored bodies similar to or identical with the same colored bodies in the cells of *Hydra fusca*. This brownish color is masked in *Hydra viridis* by the green color of the algæ.

Interesting as this suggestion is, the evidence from the colorless hydras is opposed to it. The *Hydra viridis* that has lost its green color in the glycerine solution is smaller in size, has shorter tentacles, produces fewer buds at any one time than *Hydra fusca*. It has a very faint tint of pink or brown color while *Hydra fusca* which has been in a glycerine solution 2–5 weeks has a reddish orange color.

Summary.

1. When *Hydra viridis* is kept in a .5–1.5 per cent. solution of glycerine it loses all its green color.

2. The green alga passes out of the endoderm cells into the digestive cavity from which it is expelled through the mouth to the outside when the hydra contracts.

3. The green alga does not continue to live outside in the glycerine solution.

4. The white hydras will live at least seventeen days without food.

5. The white hydras if fed can produce buds at the same rate and in the same manner as green hydras and have been kept alive for more than two months.

6. The white hydras do not show a trace of green color, under the lens, after seven weeks feeding upon contaminated food. These hydras were kept in spring water during the first 2–3 weeks of feeding and then placed in a large balanced aquaria which contained many hundreds of green hydras and much algæ. Thus showing that the white hydras were not reinfected under very favorable conditions.

7. The white hydra is positive heliotropic like the green hydra in moderate illumination.

8. The white hydra does not resemble *Hydra fusca* but, except in color, retains all the specific characters of *Hydra viridis* from which it is derived.

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